Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information for

"Hard X-ray-induced optical luminescence via biomolecule-directed metal clusters"

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Experimental section.

Materials.

DNA oligonucleotide was synthesized and purified at the Protein and Nucleic Acid Facility at Stanford University or Bex Co. (Tokyo, Japan). Lysozyme VI (Lyz) was purchased from Fisher Scientific (Chicago, IL, USA). Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), gold (Au, III) chloride hydrate, bovine serum albumin (BSA), and other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were used as received.

Synthesis of biomolecule-directed metal clusters.

Biomolecule-directed metal clusters were synthesized using slightly modified methods from previous literature.[1-4] First, all glassware was cleaned for a couple of hours in a 1.25 M sodium hydroxide (NaOH) in 40% ethanol bath and then autoclaved. Typically, for single strand DNA (ssDNA)-directed Ag clusters, the $AgNO_3$ solution (1 mM, 30 μL) was added to 200 μl of oligonucleotide solution (200 μM, 5'-CCCTTAATCCCC-3') in 200 mM sodium phosphate buffer (pH6.6) on ice. After 10 min, the mixture was reduced by addition of $NaBH_4$ aqueous solution (2 mM, 15 μL) and stored in the dark overnight at 4°C. For the Lyz-directed Au clusters, the $HAuCl_4$ solution (10 mM, 5 mL) was added to 5 mL of Lyz solution (25 mg/mL) in 0.1 M acetic acid (pH 3.0) at 37°C. The mixture was incubated overnight at 37°C. For BSA-directed Au clusters, $HAuCl_4$ aqueous solution (20 mM, 5 mL) was added to the 5 mL of BSA solution (50 mg/mL) in water at 37°C. Then, NaOH aqueous solution (1 M, 1 mL) was added to the mixture and the mixture was incubated overnight at 37°C. These samples were used for measurements without further purification.

Characterization of bio-molecule directed meal clusters.

A trans-illuminator (365 nm) was used for UV irradiation to observe luminescence from biomolecule-directed metal clusters in bulk solution. The sample solution was placed in glass bottom dish and the image was taken under UV excitation. Excitation and luminescence spectra were measured with a JASCO FP-6500 fluorometer using a micro-cuvette. The temperature was kept at 7°C for the measurement of ssDNA-Ag clusters and at room temperature for the Lys and BSA-Au clusters, respectively. Absolute quantum yield was measured using Horiba quantaϕ at room temperature. The absolute photoluminescence quantum yields (QYs) were determined using a Fluolog(r)-3 fluorescence-spectrometer (FL3-2iHR) manufactured by HORIBA Jobin Yvon equipped with a PMT (R2658 in the range of UV-Vis-NIR) and InGaAs (Symphony II IGA in the range of NIR-IR) photodetectors, a monochromator, an integrating sphere, and a 450 W xenon lamp. The emission spectra obtained were corrected using a response file that records the sensitivity of the detectors to different wavelengths of light generated using a standard lamp.

X-ray experiments.

X-ray radio-luminescence imaging was performed as previously described.[5-7] In brief, the X-ray source (Therapax SXT 150, Elimpex) voltage and current were set to 50 kV and 30 mA, respectively, and the beam was filtered with 0.4 mm aluminum foil. X-ray luminescence was measured with an electron-multiplying CCD (EM-CCD) camera (ImagEM C9100-13, Hamamatsu) using an *f*/0.95 lens (DO-1795, Navitar), 256 × 256 pixels, an exposure time of 10 s, and an EM gain of 100. The image was processed with MATLAB software to obtain the luminescence intensity data using water as reference. The relative signal intensity was shown as

1 for the H_2O reference. For the spectrum measurement, a monochromator (Princeton Instruments, Acton SP2150) was used with an exposure time and EM gain of 20 s and 100, respectively.

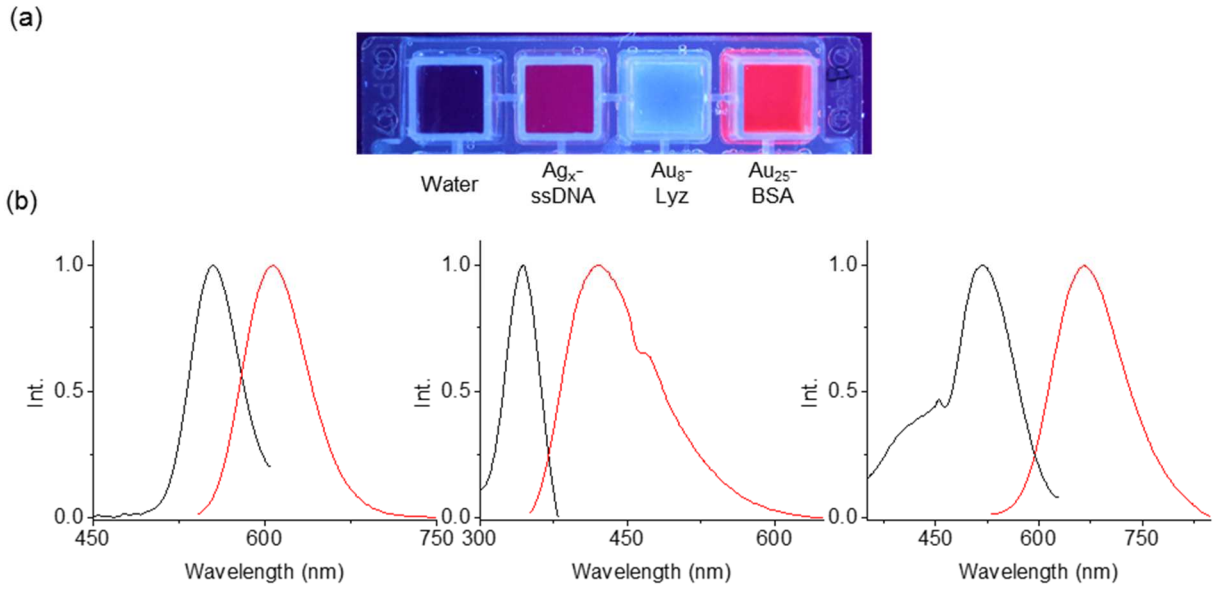

Figure S1. Steady state photo-physical properties upon UV or visible light irradiation. (a) Photograph under UV excitation; from left, **Ag$_x$-ssDNA** (pink), **Au$_8$-Lyz** (blue), and **Au$_{25}$-BSA** (pink) clusters. (b) Excitation and luminescence spectra. From left, **Ag$_x$-ssDNA**, **Au$_8$-Lyz** and **Au$_{25}$-BSA** clusters.

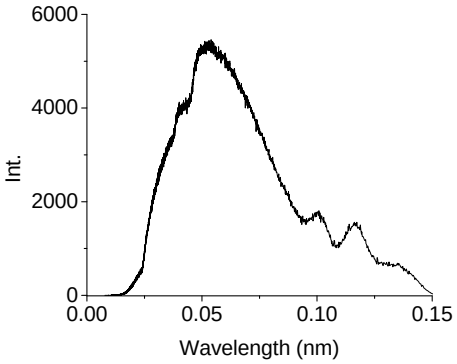

Figure S2. X-ray spectrum for the polychromatic source used in this study (50 kVp energy).

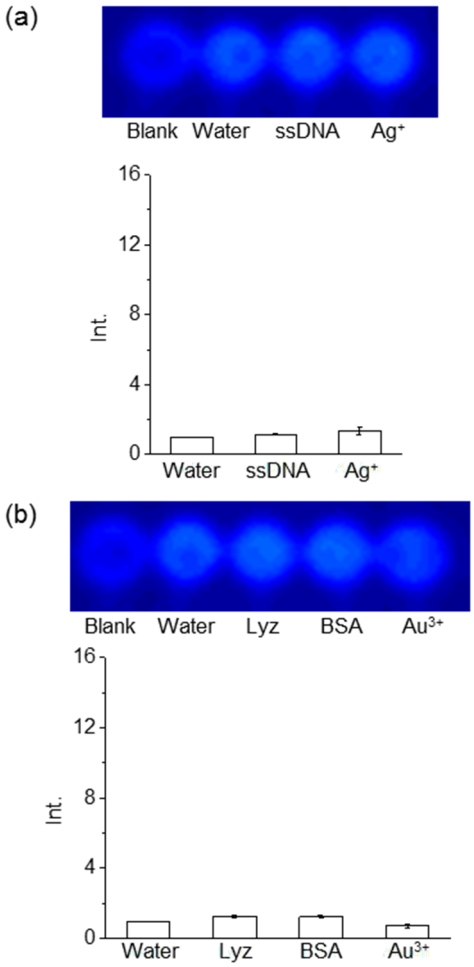

Figure S3. *h*XEOL imaging in solution of metal ions and biomolecules. (a) Images and relative luminescence intensity upon X-ray irradiation; from left, blank, water, ssDNA, and Ag ion. (b) Images and relative luminescence intensity upon X-ray irradiation; from left, blank, water, Lyz, BSA and Au ion.

Table S1. Steady state absolute quantum yields (Φ) of luminescence from bio-molecule directed metalclusters synthesized in this study. The excitation wavelength was 550 nm (Ag_x-**ssDNA**), 360 nm (Au_8-**Lyz**), and 500 nm (Au_{25}-**BSA**), respectively. Estimated error was ±20%.

	Ag_x-ssDNA	**Au_8-Lyz**	**Au_{25}-BSA**
Φ	0.4	0.06	0.04

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